

Amendments to the Specification:

Please insert on page 1, following the title "ENHANCED HIV-1 VACCINES AND METHODS FOR THEIR USE" and before "FIELD OF INVENTION" the following paragraph:

--This application is a U.S. National Phase of PCT/US2004/009617, filed March 29, 2004, which claims the benefit of U.S. provisional patent application No. 60/459,507, filed on March 31, 2003, the disclosures of which are incorporated by reference herein in their entirety.--

Please replace paragraph [07] beginning at page 3, line 1, with the following rewritten paragraph:

--[07] Accordingly, the present invention provides immunostimulating peptides having an amino acid sequence X_1 LYQYMDDV (SEQ ID NO:1), where X_1 is any hydrophobic amino acid. This amino acid sequence motif, which to our knowledge is not found in nature, is common to all peptides and proteins of the invention, and preferably has the amino acid sequence VLYQYMDDV (SEQ ID NO:2) or YLYQYMDDV (SEQ ID NO:3).--

Please replace paragraph [08] beginning at page 3, line 6, with the following rewritten paragraph:

--[08] As noted above, immunostimulating proteins are also provided by the present invention, for example, one embodiment provides an immunostimulating peptide or protein comprising the sequence X_1X_2 LYQYMDDV X_3 (SEQ ID NO:4) where X_1 is a sequence of amino acid residues of between 0 and 200 residues in length; X_2 is any hydrophobic amino acid; and, X_3 is a sequence of amino acid residues of between 0 and 200 residues in length. Preferably, these immunostimulating proteins have the sequence X_1 VLYQYMDDV X_3 (SEQ ID NO:5), or X_1 YLYQYMDDV X_3 (SEQ ID NO:6).--

Please replace paragraph [09] beginning at page 3, line 13, with the following rewritten paragraph:

--[09] A further embodiment provides proteins and fusion molecules having the amino acid sequence motif X₁LYQYMDDV (SEQ ID NO:1), where X₁ is any hydrophobic amino acid. The fusion molecules may include, for example, HIV-1 viral proteins, glycolipid conjugates, or conjugation of a protein or peptide having the X₁LYQYMDDV (SEQ ID NO:1) sequence motif with an immunostimulating carrier protein, as described herein. In some embodiments, the fusion molecules include repeat ("concatameric") X₁LYQYMDDV (SEQ ID NO:1) sequences. Concatamers of the present invention are particularly efficient both as protein/peptide antigens and, when provided as encoding nucleic acid sequences, gene therapy reagents[.], as described herein.--

Please replace paragraph [18] beginning at page 5, line 12, with the following rewritten paragraph:

--[18] Figure 1A is a comparison of the HLA-A2 binding curves among the wild type RT (179-187), VIYQYMDDL (SEQ ID NO:7), RT-1Y (YIYQYMDDL; SEQ ID NO:8), RT-2L9V (~~VL~~YQYVDDV VLYQYMDDV; SEQ ID NO:2), and RT-1Y2L9V (YLYQYMDDV; SEQ ID NO:3) in the T2-binding assay.--

Please replace paragraph [19] beginning at page 5, line 15, with the following rewritten paragraph:

--[19] Figure 1B is a comparison of the HLA-A2 binding curves among the RT-2L9V, p17-WT (SLYNTVATL; SEQ ID NO:9), RT-1Y2L9V and FMP (GILGFVFTL; SEQ ID NO:10).--

Please replace paragraph [50] beginning at page 11, line 9, with the following rewritten paragraph:

--[50] The present invention provides immunostimulatory peptides and proteins, and the nucleic acids encoding them, for use as therapeutic and diagnostic tools for the treatment of HIV infection. The peptides and proteins of the invention all share the same amino acid sequence motif, which is a variant of a synthetic sequence motif derived from the HIV-1 reverse transcriptase catalytic site region. This motif has the sequence $X_1LYQYMDDV$ (SEQ ID NO:1), where X_1 is any hydrophobic amino acid.--

Please replace paragraph [54] beginning at page 12, line 8, with the following rewritten paragraph:

--[54] The present invention provides immunostimulating peptides with the amino acid sequence $X_1LYQYMDDV$ (SEQ ID NO:11), where X_1 is any hydrophobic amino acid, preferably valine. These immunostimulatory peptides may be synthesized by any of the techniques that are known to those skilled in the peptide art, including recombinant DNA techniques and isolated natural sources, such as whole viruses or tumors, which express proteins that include a segment having the amino acid sequence of the present invention.--

Please replace paragraph [59] beginning at page 14, line 6, with the following rewritten paragraph:

--[59] One fusion molecule embodiment is a peptide or protein that includes in its amino acid sequence the sequence motif $X_1X_2YQYMDDVX_3$ (SEQ ID NO:4), where X_1 is a sequence of amino acid residues of between 0 and 200 residues in length; X_2 is any hydrophobic amino acid; and, X_3 is a second sequence of amino acid residues of between 0 and 200 residues in length that may be different from the X_1 sequence.--

Please replace paragraph [60] beginning at page 14, line 11, with the following rewritten paragraph:

--[60] Another fusion molecule embodiment of the invention can be a glycoprotein, lipoprotein, nucleoprotein or other heterologous molecule having the sequence motif $X_1LYQYMDDV$ (SEQ ID NO:1), where X_1 is any hydrophobic amino acid. Preferably this fusion protein will include an amino acid sequence for an HIV-1 viral protein or an immunostimulating carrier protein.--

Please replace paragraph [63] beginning at page 15, line 4, with the following rewritten paragraph:

--[63] The peptides of the invention may also be modified by extending their amino acid sequence, e.g., by the addition of amino acids to their N or C terminus. The peptides or fusion molecules of the invention can also be modified by altering the order or composition of certain residues, it being readily appreciated that the core immuostimulatory sequence, $X_1LYQYMDDV$ (SEQ ID NO:1), may generally not be altered without an adverse effect on biological activity. The noncritical amino acids need not be limited to those naturally occurring in proteins, such as L- α -amino acids, or their D-isomers, but may include non-natural amino acids as well, such as β - γ - δ -amino acids, as well as many derivatives of L- α -amino acids.--

Please replace paragraph [105] beginning at page 29, line 6, with the following rewritten paragraph:

--[105] Figure 1A is a comparison of the HLA-A2 binding curves among the wild type RT (179-187), $VIYQYMDDL$ (SEQ ID NO:7), RT-1Y ($YIYQYMDDL$; SEQ ID NO:8), RT-2L9V

(VL~~Y~~Q~~Y~~V~~D~~D~~V~~ VL~~Y~~Q~~Y~~M~~D~~D~~V~~; SEQ ID NO:2), and RT-1Y2L9V (YL~~Y~~Q~~Y~~M~~D~~D~~V~~; SEQ ID NO:3) in the T2-binding assay.--

Please replace paragraph [106] beginning at page 29, line 9, with the following rewritten paragraph:

--[106] Figure 1B compares HLA-A2 binding curves among the RT-2L9V, p17-WT (SLYNTVATL; SEQ ID NO:9), RT-1Y2L9V and FMP (GILGFVFTL; SEQ ID NO:10).--

Please replace paragraph [112] and Table 2 beginning at page 30, line 29, with the following rewritten paragraph:

--[112] Binding affinity of wild type RT (179-187) (RT-WT) using the T2 binding assay, measuring the cell surface stabilization of HLA-A2.1 molecules after incubation with peptide. Relative affinity for MHC molecules was determined for each peptide in ~~table~~ Table 2 by comparing their FI_{0.5} values as calculated from titration curves against HLA-A2 molecules. Using this method, an FI_{0.5} of 41.9 μ M was calculated for RT-WT. This binding affinity was much weaker than that of other 9-mer peptides tested in our lab such as hepatitis C virus peptide C7A2 (Sarobe, P., *et al.*, (1998) *J.Clin.Invest.* 102:1239), Flu matrix peptide 58-66 (FMP) (Gotch, F. M., *et al.*, (1987). *Nature* 326:881.), and HIV-gag peptide SLYNTVATL (SEQ ID NO:9) (McMichael, A. J., and B. D. Walker. (1994), *AIDS* 8 (*suppl 1*):S155; See also ~~table~~ Table 2). In a set of experiments to define key functional residues, peptides with alanine substitutions at each one of the positions were synthesized and tested in binding assays, as described above. The results of these experiments are summarized in ~~table~~ Table 2, below.

Table 2. Binding of RT (179 –187)-wild type and –substituted peptide to HLA-A2.

Peptide	Sequence	<u>SEQ ID NO:</u>	FI _{0.5}
RT (179 – 187)-WT	VIYQYMDDL	<u>7</u>	41.9
1A	AIYQYMDDL	<u>12</u>	33.7
2A	VAYQYMDDL	<u>13</u>	> 100
3A	VIAQYMDDL	<u>14</u>	41.2
4A	VIYAYMDDL	<u>15</u>	40.7
5A	VIYQAMDDL	<u>16</u>	95.6
6A	VIYQYADDL	<u>17</u>	17.4
7A	VIYQYMADL	<u>18</u>	> 100
8A	VIYQYMDAL	<u>19</u>	35.9
9A	VIYQYMDDA	<u>20</u>	57.9
2L	VLQYQYMDDL	<u>21</u>	19.2
9V	VIYQYMDDV	<u>22</u>	19.9
2L9V (RT-2L9V)	VLQYQYMDDV	<u>2</u>	5.7
gag (p17) (77-85)	SLYNTVATL	<u>9</u>	2.21
Flu-MP (58-66)	GILGFVFTL	<u>10</u>	0.24

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Please replace paragraph [115] beginning at page 32, line 19, with the following rewritten paragraph:

--[115] Recent studies reported that a Tyrosine substitution in the first position (P¹Y) can increase peptide/MHC binding without altering antigenic specificity (Pogue, R. R., *et al.*, (1995) Proc.Natl.Acad.Sci.U.S.A. 92:8166; Tourdot, S., A. *et al.*, (2000) Eur J Immunol 30:3411). Based on these studies, we used the T2 binding assay to compare peptide/MHC binding among 4 derivative peptides:

RT-WT,
RT-2L9V,
RT-1Y (YIYQYMDDL; SEQ ID NO:8), and
RT-1Y2L9V (YLYQYMDDV; SEQ ID NO:3)--

Appl. No. 10/551,405
Amdt. dated August 21, 2008
Reply to Office Action of June 3, 2008

PATENT

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1-11, at the end of the application.